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UBIQUITIN-SPECIFIC PROTEASE16 interacts with a HEAVY METAL ASSOCIATED ISOPRENYLATED PLANT PROTEIN27 and modulates cadmium tolerance

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Protein ubiquitination and deubiquitination are two reversible processes catalyzed by ubiquitin ligases and deubiquitinating enzymes, respectively. In *Arabidopsis*, lots of substrates of ubiquitin ligases were found, whereas only a few targets of deubiquitinating enzymes were identified. Recently, we reported that a functional UBIQUITIN-SPECIFIC PROTEASE16 (UBP16) was involved in salt tolerance through positively regulating plasma membrane Na^+/H^+ antiport activity and at least partially modulating SERINE HYDROXYMETHYLTRANSFERASE1 (SHM1) stability and activity. Here, we report that UBP16 interacts with HEAVY METAL ASSOCIATED ISOPRENYLATED PLANT PROTEIN27 (HIPP27), a metallochaperone containing a predicted heavy-metal-associated domain, which has been reported to play an important role in cadmium detoxification. Meanwhile, the *ubp16* mutant showed more sensitive to cadmium than wild-type. Taken together, HIPP27 may be another target of UBP16 in cadmium response.

Modification of protein by ubiquitin was widely distributed in eukaryotes, which was sequentially catalyzed by E1 ubiquitin-activating enzyme, E2 ubiquitin-conjugating enzyme and E3 ubiquitin-protein ligase.[1,2] In *Arabidopsis*, more than 1 400 ubiquitin ligases were identified, which target specific substrates.[3] Deubiquitinating enzymes (DUBs) are proteases that reverse the modification of proteins by ubiquitin. DUBs are divided into two general groups, ubiquitin C-terminal hydrolases (UCHs) and ubiquitin-specific proteases (UBPs) based on their amino acid sequence and substrate specificity.[4,5] Estimated in a recent phylogenetic analysis, there are at least 64 UCHs and 27 UBPs in *Arabidosis*.[6] The number of DUBs is significantly less than that of ubiquitin ligase, suggesting that one DUB may target different substrates under variant conditions.

Recently, though systemic analysis of UBPs family members responsive to salt tolerance, we found the *ubp16* mutant was more sensitive to salt than wild-type and other UBPs mutants. UBP16 positively regulates plasma membrane Na^+/H^+ antiport activity and the *ubp16* mutant accumulated more sodium. Through yeast two-hybrid assay, we identified a putative target of UBP16, SHM1. The stability and activity of SHM1 were changed in the *ubp16* mutant, resulting in accelerated cell death and accumulation of reactive oxygen species. Taken together, UBP16 was involved in salt tolerance in Arabidopsis by regulating Na^+/H^+ antiport activity and repressing cell death partially through regulating SHM1 stability and activity.[7] Besides SHM1, we obtained another positive clone from Arabidopsis cDNA library through yeast two-hybrid (**Fig. 1**). Sequence analysis revealed that this clone harbored the full-length coding sequence of HIPP27, which belongs to the metallochaperone-like proteins that traffic metal ion within cells and sequester metals in cell compartment.[8]

Cadmium (Cd) is a widespread non-essential toxic heavy metal and considered as a serious environmental pollutant. Cadmium has adverse effects on plant development such as inhibition of root growth and induction of chlorosis in leaves.[9] In Arabidopsis, it was reported that HIPP20, HIPP22, HIPP26, and HIPP27 played an important role in Cd-detoxification.[8] In order to determine whether UBP16 is involved in Cadmium tolerance, we transferred 7-d-old seedlings grown on MS medium to MS medium with or without 30 μM $CdCl_2$ for 12 d. In the absence of $CdCl_2$, there was no significant difference in shoot growth and anthocynanin contents between wild-type and the *ubp16* mutant

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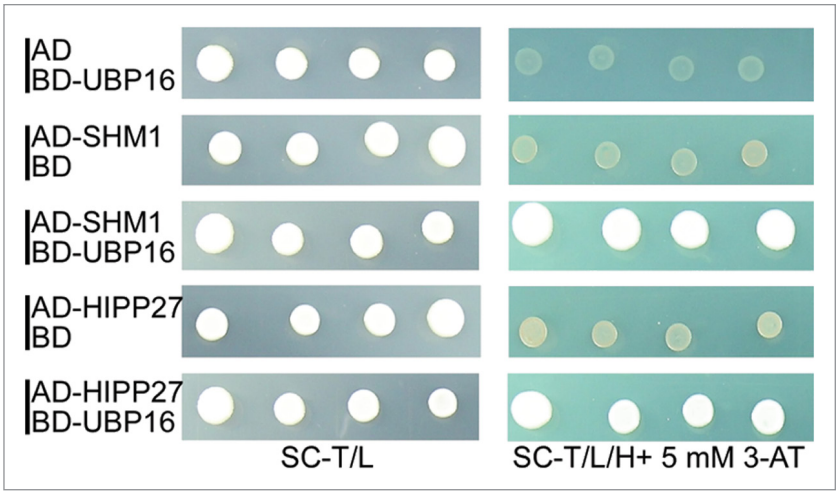

Figure 1. UBP16 interacts with HIPP27 through yeast two-hybrid. Yeast strains containing AD-HIPP27 and BD-UBP16 grew better than that harboring AD and BD-UBP16 or AD-HIPP27 and BD vector on synthetic complete (SC) medium without Trp, Ler and His, 5 mM 3-AT plus. SHM1 as positive control.

seedlings. However, in the presence of 30 μM $CdCl_2$, shoot growth of the *ubp16* mutant was more severely impaired than wild-type (**Fig. 2**), and the anthocyanin contents of the *ubp16* mutant was significant higher than that of wild-type, suggesting that the *ubp16* mutant is more sensitive to $CdCl_2$. In summary, these evidences implied that HIPP27 may be another target of UBP16 and their interaction modulates Cadmium tolerance, although other evidences are needed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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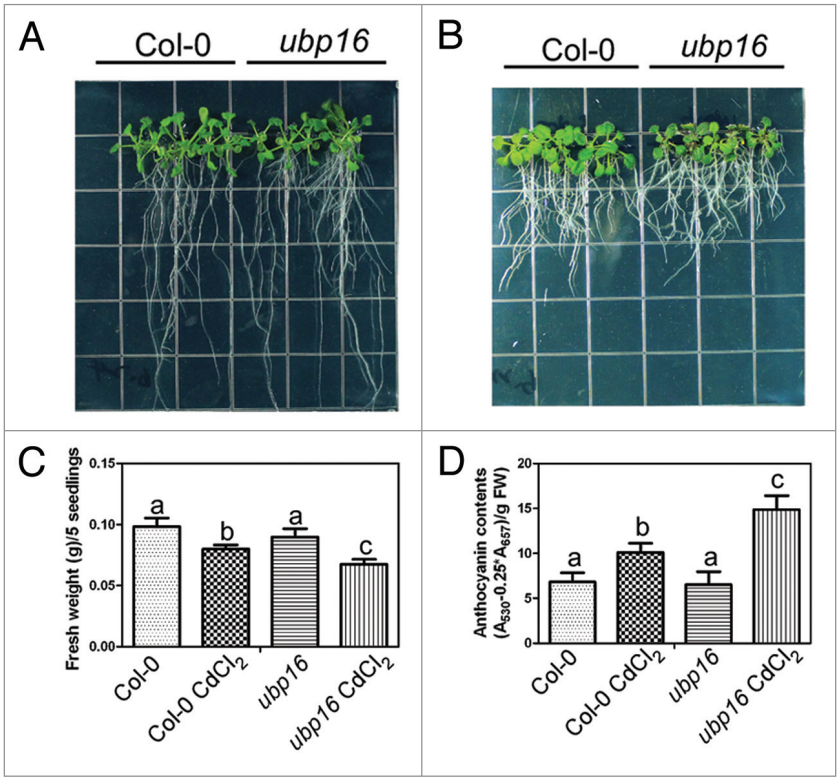

Figure 2. Analysis of Cadmium sensitivity in wild-type (Col-0) and the *ubp16* mutant seedlings. (**A**) and (**B**) Col-0 and the *ubp16* seedlings grown on MS medium with or without 30 μM $CdCl_2$. Photograph were taken 12 d after transfer. (**C**) Fresh weight. (**D**) Anthocyanin contents. Error bars represent SD (n > 6). Statistical significance was determined by the Student's t test.

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